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## Data Article

# Characterization of human T cell receptor repertoire data in eight thymus samples and four related blood samples



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## ABSTRACT

T cell receptor (TCR) is a heterodimer consisting of TCR $\alpha$  and TCR $\beta$  chains that are generated by somatic recombination of multiple gene segments. Nascent TCR repertoire undergoes thymic selections where non-functional and potentially autoreactive receptors are removed. During the last years, the development of high-throughput sequencing technology has allowed a large scale assessment of TCR repertoire and multiple analysis tools are now also available.

In our recent manuscript, *Human thymic T cell repertoire is imprinted with strong convergence to shared sequences* [1], we show highly overlapping thymic TCR repertoires in unrelated individuals. In the current Data in Brief article, we provide a more detailed characterization of the basic features of these thymic and related peripheral blood TCR repertoires. The thymus samples were collected from eight infants undergoing corrective cardiac surgery, two of whom were monozygous

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twins [2]. In parallel with the surgery, a small aliquot of peripheral blood was drawn from four of the donors. Genomic DNA was extracted from mechanically released thymocytes and circulating leukocytes. The sequencing of TCR $\alpha$  and TCR $\beta$  repertoires was performed at ImmunoSEQ platform (Adaptive Biotechnologies). The obtained repertoire data were analysed applying relevant features from immunoSEQ® 3.0 Analyzer (Adaptive Biotechnologies) and a freely available VDJTools software package for programming language R [3]. The current data analysis displays the basic features of the sequenced repertoires including observed TCR diversity, various descriptive TCR diversity measures, and V and J gene usage. In addition, multiple methods to calculate repertoire overlap between two individuals are applied. The raw sequence data provide a large database of reference TCRs in healthy individuals at an early developmental stage. The data can be exploited to improve existing computational models on TCR repertoire behaviour as well as in the generation of new models.

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Specifications Table

Subject	Immunology
Specific subject area	T cell antigen receptor (TCR) alpha chain and beta chain diversity and characteristics in thymus and in peripheral blood
Type of data	Table: Sample description by immunoSEQ and VDJTools softwares (Table 1), repertoire diversity metrics (Table 2), resampled repertoire diversity metrics (Table 3), repertoire overlap measures (Table 4). Graph: V gene usage heatmap (Figure 1), J gene usage heatmap (Figure 2), rarefaction plots (Figure 3), clustering of overlap analyses (Figure 4).
How data were acquired	TCRAD and TCRB sequencing was performed at ImmunoSEQ platform (Adaptive Biotechnologies). TCR analysis was performed using immunoSEQ® 3.0 Analyzer (Adaptive Biotechnologies) and VDJTools software [3].
Data format	Raw Analysed
Parameters for data collection	Thymus samples were obtained from eight immunologically healthy infants undergoing open cardiac surgery for congenital heart defects. A small aliquot of blood (0.5–1 mL) was drawn from four subjects during the operation. The study was approved by the Pediatric Ethical Committee of the Helsinki University Hospital (HUS/747/2019) and a written informed consent was obtained from the parents.
Description of data collection	Thymocytes were extracted mechanically from tissue resects. Blood samples were treated with ACK lysis buffer (Thermo Fisher Scientific) to remove erythrocytes. DNA was extracted from 10–30 million thymocytes and from all available PBMCs. TCRAD and TCRB sequencing was performed as previously described [4] from a standardized quantity of genomic DNA using ImmunoSEQ assay (Adaptive Biotechnologies), which exploits a multiplex PCR system spanning the V(D)J region at a length that is sufficient to identify V and J genes and cover unique CDR3 regions.
Data source location	Institution: University of Helsinki City/Town/Region: Helsinki Country: Finland
Data accessibility	Repository name: The European Nucleotide Archive (ENA) at EMBL-EBI Data identification number: PRJEB41936 Direct URL to data: <a href="https://www.ebi.ac.uk/ena/browser/view/PRJEB41936">https://www.ebi.ac.uk/ena/browser/view/PRJEB41936</a>

(continued on next page)

## Related research article

Heikkilä Nelli, Vanhanen Reetta, Yohannes Dawit A., Kleino Iivari, Mattila Ilkka P., Saramäki Jari, Arstila T. Petteri  
 Human thymic T cell repertoire is imprinted with strong convergence to shared sequences  
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## Value of the Data

- These data consist of a unique collection of over 62 million T cell receptor (TCR) sequences obtained directly from human thymus. It is a large scale resource of human TCR $\alpha$  and TCR $\beta$  repertoires at an early developmental stage before clonal selections by peripheral antigens and devoid of medical or immunological interventions.
- The data are useful for those who wish to compare TCR repertoires from healthy thymus and from individuals affected by immunological diseases or other medical conditions. The large scale thymic repertoire data can also benefit computational experiments which have been typically limited to peripheral blood TCR data.
- These data can be directly exploited to improve existing computational models on TCR repertoire generation as well as in the generation of new models. These data can also guide design of human TCR sequencing experiments and serve as a reference database for new experiments.

## 1. Data Description

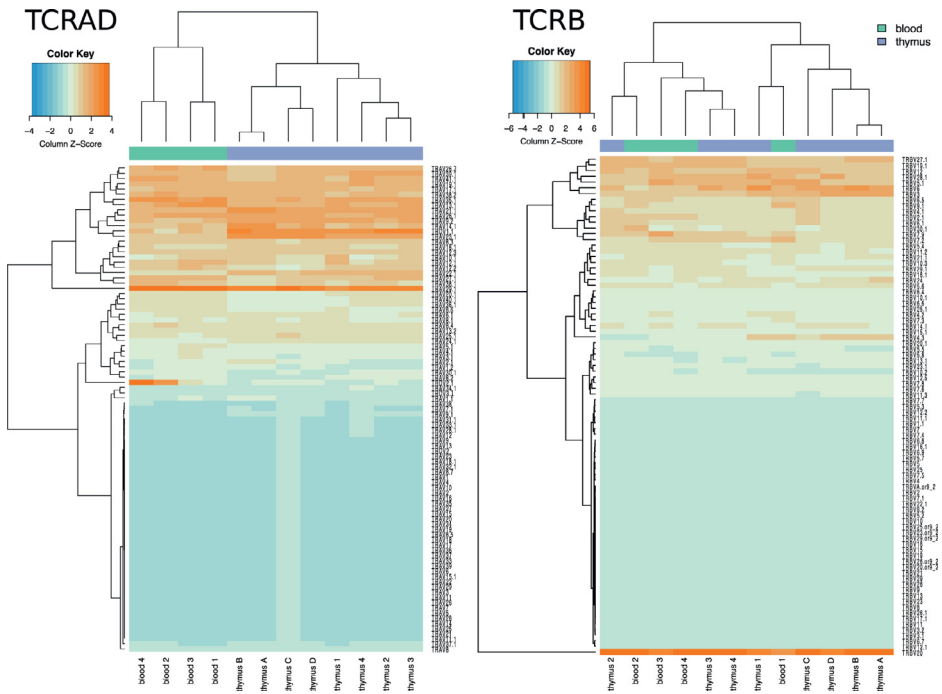
All TCRAD and TCRB sequences obtained from eight thymus (donors A-D and donors 1–4) and four related blood samples (donors 1–4) have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under accession number PRJEB41936 (<https://www.ebi.ac.uk/ena/browser/view/PRJEB41936>). In addition, the sequences are available at immuneACCESS<sup>®</sup> repository in the form of immunoSEQ<sup>™</sup> output format and can also be downloaded as raw FASTA files (<https://clients.adaptivebiotech.com/pub/heikkila-2020-mi>). On average, we obtained 4.1 million unique TCR $\alpha$  and 810 000 unique TCR $\beta$  clonotypes from each thymus. From blood samples we obtained on average 150 000 and 84 000 unique TCR $\alpha$  and TCR $\beta$  sequences, respectively. An overview of sequence diversities, total counts and sequence productivity (in-frame vs. non-coding sequences) was generated both by immunoSEQ<sup>™</sup> and VDJTools softwares and is displayed together with donor details in Table 1. Two of the donors (A and B) were monozygous twins and the influence of genetics in the repertoire has been analysed previously [2]. The V and J gene usage has been shown to be biased in the peripheral blood but also already in the thymus [5–7]. The gene segment usage in the current samples is also biased (Figs. 1 & 2).

The TCR diversity has been previously assessed both in the peripheral blood and in the thymus and multiple diversity metrics are available [4,8–10]. The diversity estimates for the current samples were calculated using VDJTools software with default settings. To estimate the lower bound of total species richness, VDJTools provide unmodified Chao1, extrapolated Chao (chaoE) and Efron-Thisted estimates while the repertoire diversity is depicted with Shannon's index and inverse Simpson's index (Table 2). The species richness and repertoire diversity indexes are also calculated for datasets down-sampled to the size of the smallest dataset to facilitate the comparison of samples with different sequencing depths (Table 3). Furthermore, a rarefaction curve based on the relationship between the sample diversity and the sample size was plotted for TCR $\alpha$  and TCR $\beta$  with extrapolation to the size of the largest sample (Fig. 3).

Despite the high potential diversity of TCR repertoires, a surprisingly high fraction of the repertoire is shared between individuals [1]. Here, we calculated various overlap measures with VDJTools: Pearson correlation, relative overlap measure [rationale explained in 11], Jaccard index

**Table 1**  
Description of the sequenced samples.

TCRAD											
Sample id	Age (days)	Sex	Tissue	immunoSEQ™: count	VDJTools: count	immunoSEQ™: diversity	VDJTools: diversity	immunoSEQ™: non-coding diversity	VDJTools: non-coding diversity	immunoSEQ™: non-coding frequency	VDJTools: non-coding frequency
thymus A	243	M	thymus	11 838 086	11 838 086	6 907 422	6 763 870	4 719 902	2 090 241	68.33%	31.10%
thymus B	244	M	thymus	12 849 473	12 849 473	7 578 104	7 419 245	5 179 754	2 307 719	68.35%	31.22%
thymus C	225	F	thymus	8 359 283	8 359 283	5 347 824	5 259 057	3 663 398	1 752 208	68.50%	33.98%
thymus D	126	M	thymus	11 063 464	11 063 464	6 743 495	6 610 182	4 617 533	2 007 905	68.47%	30.29%
thymus 1	7	M	thymus	3 179 774	3 179 774	2 089 557	1 984 292	1 447 726	601 486	69.28%	30.01%
thymus 2	52	M	thymus	1 747 487	1 747 487	1 262 845	1 198 677	883 536	385 749	69.96%	32.05%
thymus 3	107	M	thymus	2 158 043	2 158 043	1 289 728	1 230 436	902 934	398 227	70.01%	32.24%
thymus 4	156	F	thymus	1 848 851	1 848 851	1 419 013	1 345 927	997 764	441 964	70.31%	32.71%
blood 1	7	M	blood	154 682	154 682	138 159	130 307	86 201	34 147	62.39%	26.02%
blood 2	52	M	blood	123 523	123 523	109 171	103 142	65 096	26 413	59.63%	25.12%
blood 3	107	M	blood	245 126	245 126	180 100	170 333	110 571	45 852	61.39%	24.94%
blood 4	156	F	blood	199 326	199 326	167 266	157 728	104 846	45 404	62.68%	27.89%
TCRB											
Sample id	Age (days)	Sex	Tissue	immunoSEQ™: count	VDJTools: count	immunoSEQ™: diversity	VDJTools: diversity	immunoSEQ™: non-coding diversity	VDJTools: non-coding diversity	immunoSEQ™: non-coding frequency	VDJTools: non-coding frequency
thymus A	243	M	thymus	1 647 656	1 647 656	1 254 760	1 245 029	288 199	108 933	22.97%	8.46%
thymus B	244	M	thymus	1 783 878	1 783 878	1 540 161	1 526 694	363 551	138 386	23.60%	8.89%
thymus C	225	F	thymus	1 850 299	1 850 299	1 568 528	1 551 603	248 898	93 724	15.87%	5.89%
thymus D	126	M	thymus	1 726 796	1 726 796	1 462 150	1 449 881	279 672	106 019	19.13%	7.15%
thymus 1	7	M	thymus	237 063	237 063	223 725	222 925	53 389	19 585	23.86%	8.76%
thymus 2	52	M	thymus	182 356	182 356	173 368	172 746	35 779	14 443	20.64%	8.28%
thymus 3	107	M	thymus	142 903	142 903	138 544	137 920	31 385	12 183	22.65%	8.75%
thymus 4	156	F	thymus	128 228	128 228	122 195	121 483	25 475	10 129	20.85%	8.24%
blood 1	7	M	blood	82 418	82 418	77 868	77 281	21 203	7 462	27.23%	9.51%
blood 2	52	M	blood	73 945	73 945	69 875	69 404	17 566	6 783	25.14%	9.81%
blood 3	107	M	blood	134 110	134 110	104 236	103 551	26 162	9 831	25.10%	8.73%
blood 4	156	F	blood	88 901	88 901	82 550	81 935	20 852	8 151	25.26%	10.19%



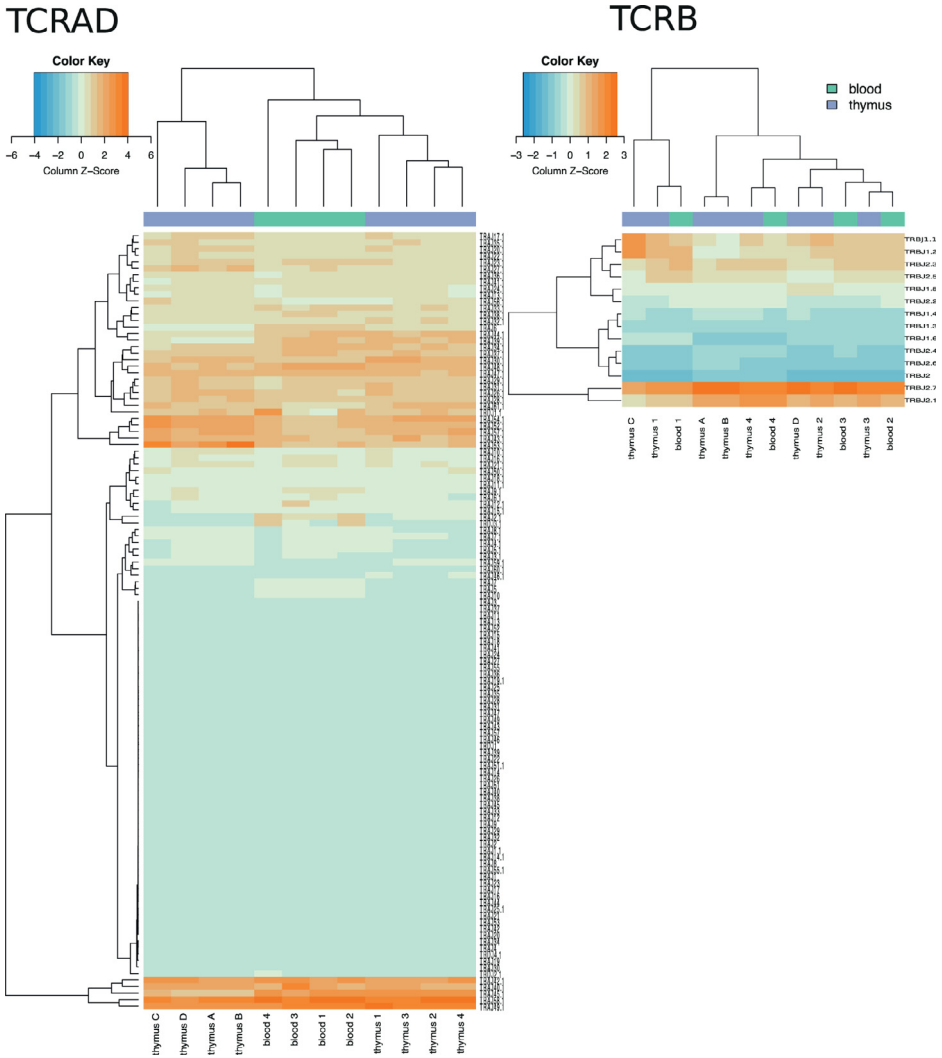
**Fig. 1.** The V gene usage in TCRAD and TCRB repertoires. Z-scores indicate the relative frequency of each segment. Dendrograms show clustering of the samples and the gene segments.

and Morisita-Horn index (Table 4). The calculations were performed on the entire repertoire and exact matching of V gene, J gene and the CDR3 region was required. The clustering of different samples with multidimensional scaling is depicted for Jaccard index (Fig. 4).

## 2. Experimental Design, Materials and Methods

Thymus samples were obtained from eight immunologically healthy infants undergoing corrective cardiac surgery for congenital heart defects. The study was approved by the Pediatric Ethical Committee of the Helsinki University Hospital (HUS/747/2019). A written informed consent was obtained from the parents. Thymocytes were extracted mechanically from tissue resects and stored as pellets of 10–30 million thymocytes in  $-70^{\circ}\text{C}$ . From four donors a small aliquot of 0.5–1 mL peripheral blood was drawn during the surgery. To remove erythrocytes, the blood samples were treated with ACK lysis buffer (Thermo Fisher Scientific, USA) according to manufacturer's orders and the obtained leukocytes were stored as pellets in  $-70^{\circ}\text{C}$ . Genomic DNA was extracted from frozen pellets with QIASymphony<sup>TM</sup> (Qiagen, Germany) according to manufacturer's orders. TCRAD and TCRB regions were sequenced from a standardized quantity of quality-controlled genomic DNA using ImmunoSEQ<sup>TM</sup> assay (Adaptive Biotechnologies). The assay uses a multiplex PCR system spanning the TCRAD VJ and TCRB VDJ regions at a length that is sufficient to cover unique CDR3 regions and to identify V and J genes. Amplicon sequencing was performed on Illumina platform. TCRAD and TCRB definitions were based on IMGT database ([www.imgt.org](http://www.imgt.org)). Primer bias and sequencing errors were corrected as previously described [4].

For each sequenced sample the ImmunoSEQ<sup>TM</sup> assay outputs a file of unique nucleotide sequences covering V and J genes and the CDR3 region, the count and frequency of each sequence, the CDR3 region length, and whether the sequence is in-frame, out-of-frame or contains a



**Fig. 2.** The J gene usage in TCRAD and TCRB repertoires. Z-scores indicate the relative frequency of each segment. Dendrograms show clustering of the samples and the gene segments.

premature STOP codon. For in-frame and ‘has stop’ sequences the nucleotide sequence is converted to CDR3 amino acid sequence and \* symbol indicates the STOP codon. In addition, the V gene, D gene and J gene names, the number of non-templated nucleotide insertions and the locations of insertions in V and J gene segments are provided. The raw FASTA files are also available but not directly used in the present analysis.

In the current article we applied TCR analysis tools from two platforms: immunoSEQ™ ANALYZER 3.0 run on Adaptive Biotech website ([adaptivebiotech.com/products-services/immunoseq/immunoseq-analyzer/](https://adaptivebiotech.com/products-services/immunoseq/immunoseq-analyzer/)) and a java based non-commercial software package VDJTools [3]. From immunoSEQ™ we adapted “Sample Overview” to calculate the sample diversity and counts. VDJTools readily accepts the basic immunoSEQ™ output format and converts it to a VDJTools output file. From VDJTools we used “CalcBasicStats” command to calculate the sample diversity and counts, “CalcSegmentUsage” command to produce V and J gene usage heatmaps,

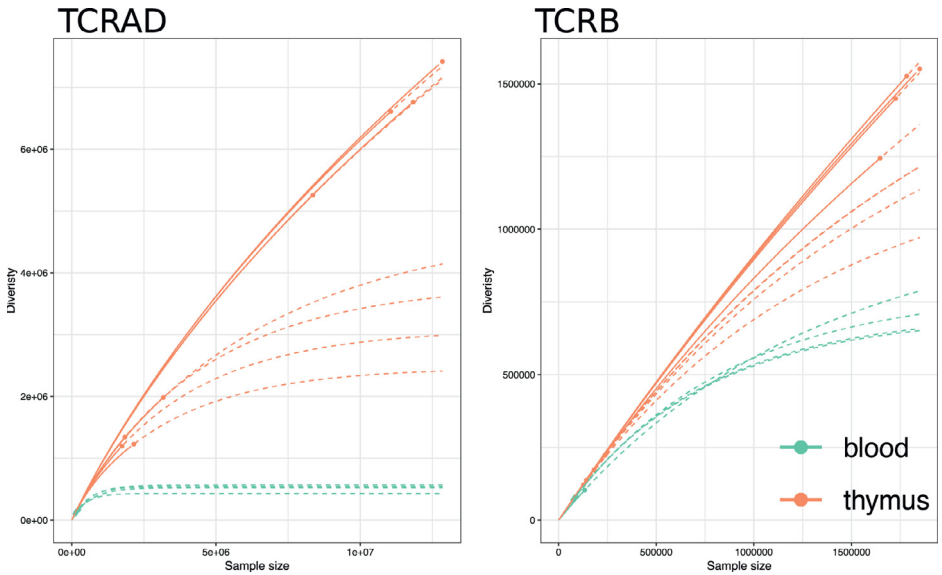
**Table 2**  
Diversity estimates.

TCRAD										
Sample id	Age (days)	Sex	Tissue	Observed counts	Observed diversity	Chao1 (mean±std)	Extrapolated ChaoE (mean±std)	Efron-Thisted (mean±std)	Shannon's index (mean)	Inversed Simpson's index (mean)
thymus A	243	M	thymus	11 838 086	6 763 870	16 826 997 ± 13 658	7 160 320 ± 2 017	46 864 937 ± 2 469 178	4 617 696	1 633 144
thymus B	244	M	thymus	12 849 473	7 419 245	18 828 064 ± 14 772	7 419 245 ± 2 120	41 786 084 ± 2 534 757	5 058 759	1 796 531
thymus C	225	F	thymus	8 359 283	5 259 057	14 602 322 ± 14 425	7 124 951 ± 2 068	45 140 688 ± 3 293 359	3 861 431	1 603 852
thymus D	126	M	thymus	11 063 464	6 610 182	16 698 624 ± 13 750	7 342 590 ± 2 021	23 898 564 ± 1 279 838	4 677 030	1 764 774
thymus 1	7	M	thymus	3 179 774	1 984 292	4 754 478 ± 6 900	4 141 693 ± 3 605	11 705 484 ± 692 435	1 588 328	1 011 926
thymus 2	52	M	thymus	1 747 487	1 198 677	3 089 405 ± 5 992	2 989 346 ± 4 808	7 000 872 ± 416 869	1 017 426	771 547
thymus 3	107	M	thymus	2 158 043	1 230 436	2 468 027 ± 3 953	2 410 929 ± 3 240	9 121 480 ± 635 237	985 201	708 503
thymus 4	156	F	thymus	1 848 851	1 345 927	3 812 418 ± 7 380	3 606 280 ± 5 422	6 826 960 ± 368 256	1 166 396	885 107
blood 1	7	M	blood	154 682	130 307	540 969 ± 4 198	540 969 ± 4 123	827 694 ± 56 037	121 367	108 361
blood 2	52	M	blood	123 523	103 142	430 223 ± 3 766	430 223 ± 3 700	681 737 ± 35 608	94 556	76 513
blood 3	107	M	blood	245 126	170 333	522 505 ± 3 003	522 505 ± 2 923	885 943 ± 47 301	114 452	5 901
blood 4	156	F	blood	199 326	157 728	56 686 ± 3 696	566 868 ± 3 615	1 270 020 ± 90 170	137 613	62 127
TCRB										
Sample id	Age (days)	Sex	Tissue	Observed counts	Observed diversity	Chao1 (mean±std)	Extrapolated ChaoE (mean±std)	Efron-Thisted (mean±std)	Shannon's index (mean)	Inversed Simpson's index (mean)
thymus A	243	M	thymus	1 647 656	1 245 029	3 463 693 ± 6 808	1,360,137 ± 899	5 568 945 ± 281 571	1 115 157	949 669
thymus B	244	M	thymus	1 783 878	1 526 694	7 198 338 ± 17 565	1,576,012 ± 1 097	11 613 883 ± 828 672	1 426 418	1 275 231
thymus C	225	F	thymus	1 850 299	1 551 603	6 175 838 ± 13 550	1 551 603 ± 1 077	11 065 398 ± 661 911	1 446 717	1 302 914
thymus D	126	M	thymus	1 726 796	1 449 881	6 208 706 ± 14 737	1 537 939 ± 1 056	9 910 366 ± 569 512	1 343 436	1 185 806
thymus 1	7	M	thymus	237 063	222 925	2 056 755 ± 18 593	1 215 349 ± 4 192	2 736 169 ± 206 873	217 609	209 474
thymus 2	52	M	thymus	182 356	172 746	1 737 599 ± 18 637	1 136 517 ± 5 332	2 180 279 ± 113 043	169 209	163 907
thymus 3	107	M	thymus	142 903	137 920	2 039 501 ± 29 774	1 215 583 ± 6 936	1 782 350 ± 140 139	136 060	133 205
thymus 4	156	F	thymus	128 228	121 483	1 269 967 ± 16 587	971 553 ± 6 988	1 569 435 ± 90 703	118 934	115 045
blood 1	7	M	blood	82 418	77 281	713 105 ± 10 949	658 522 ± 7 882	865 983 ± 57 876	75 069	70 167
blood 2	52	M	blood	73 945	69 404	704 739 ± 12 003	651 718 ± 8 696	796 697 ± 63 815	67 046	59 899
blood 3	107	M	blood	134 110	103 551	1 029 542 ± 14 292	787 853 ± 6 017	1 089 644 ± 87 860	47 612	1 278
blood 4	156	F	blood	88 901	81 935	790 042 ± 12 109	707 833 ± 7 921	898 294 ± 62 024	77 810	64 418

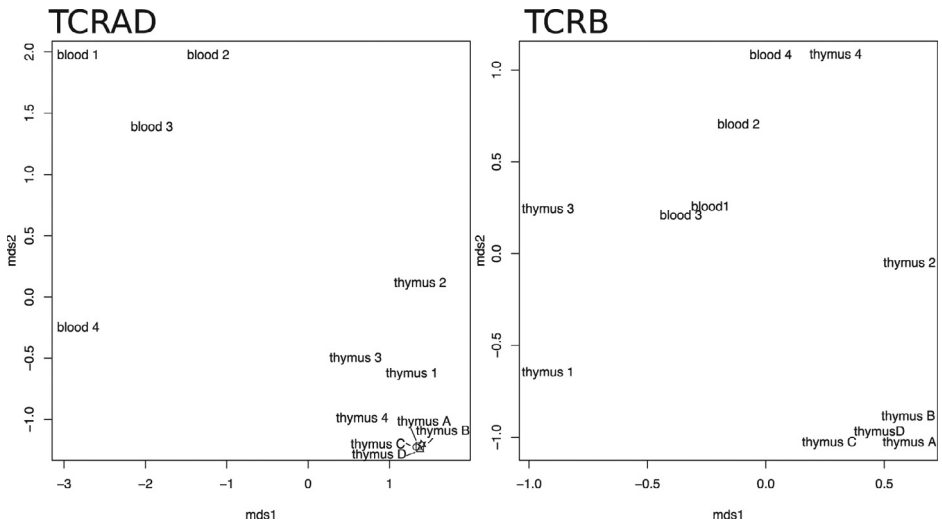


**Table 3**  
Resampled diversity estimates.

TCRAD									
Sample id	Age (days)	Sex	Tissue	Observed counts	Observed diversity	Resampled Chao1 (mean±std)	Resampled Efron-Thisted (mean±std)	Resampled Shannon's index (mean)	Resampled inversed Simpson's index (mean)
thymus A	243	M	thymus	11 838 086	6 763 870	3 439 802 ± 48 656	1 928 183 ± 109 031	119 114	116 022
thymus B	244	M	thymus	12 849 473	7 419 245	3 717 184 ± 117 571	1 934 212 ± 165 908	119 461	116 728
thymus C	225	F	thymus	8 359 283	5 259 057	3 519 847 ± 71 955	2 023 854 ± 156 215	119 278	116 379
thymus D	126	M	thymus	11 063 464	6 610 182	3 799 437 ± 74 891	2 113 509 ± 195 602	119 460	116 538
thymus 1	7	M	thymus	3 179 774	1 984 292	2 131 831 ± 30 009	1 796 761 ± 112 417	117 634	114 042
thymus 2	52	M	thymus	1 747 487	1 198 677	1 703 786 ± 14 235	1 686 940 ± 56 182	116 726	113 381
thymus 3	107	M	thymus	2 158 043	1 230 436	1 302 763 ± 10 861	1 458 706 ± 13 181	114 778	110 665
thymus 4	156	F	thymus	1 848 851	1 345 927	2 227 657 ± 27 749	1 956 797 ± 46 144	118 117	115 234
blood 1	7	M	blood	154 682	130 307	515 756 ± 2 407	850 317 ± 49 399	101 160	92 134
blood 2	52	M	blood	123 523	103 142	430 223 ± 0	681 737 ± 0	94 556	76 513
blood 3	107	M	blood	245 126	170 333	421 347 ± 1 715	760 367 ± 58 240	71 403	5 814
blood 4	156	F	blood	199 326	157 728	506 377 ± 1 192	824 158 ± 38 655	95 097	51 910
TCRB									
Sample id	Age (days)	Sex	Tissue	Observed counts	Observed diversity	Resampled Chao1 (mean±std)	Resampled Efron-Thisted (mean±std)	Resampled Shannon's index (mean)	Resampled inversed Simpson's index (mean)
thymus A	243	M	thymus	1 647 656	1 245 029	2 402 723 ± 69 516	1 320 715 ± 8 691	72 359	71 675
thymus B	244	M	thymus	1 783 878	1 526 694	4 788 673 ± 151 470	1 437 422 ± 2 372	73 122	72 751
thymus C	225	F	thymus	1 850 299	1 551 603	4 582 751 ± 147 345	1 423 151 ± 2 423	73 119	72 760
thymus D	126	M	thymus	1 726 796	1 449 881	4 058 264 ± 145 518	1 413 346 ± 5 795	72 976	72 540
thymus 1	7	M	thymus	237 063	222 925	1 912 950 ± 45 644	1 275 516 ± 15 408	71 922	71 039
thymus 2	52	M	thymus	182 356	172 746	1 663 403 ± 52 735	1 238 325 ± 16 294	71 651	70 673
thymus 3	107	M	thymus	142 903	137 920	1 974 903 ± 20 177	1 263 684 ± 15 635	72 045	71 233
thymus 4	156	F	thymus	128 228	121 483	1 206 007 ± 23 326	1 135 274 ± 12 074	70 702	69 306
blood 1	7	M	blood	82 418	77 281	709 395 ± 4 431	816 448 ± 47 909	67 932	63 859
blood 2	52	M	blood	73 945	69 404	704 739 ± 0	796 697 ± 0	67 046	59 899
blood 3	107	M	blood	134 110	103 551	895 010 ± 8 921	798 491 ± 78 565	30 118	1 268
blood 4	156	F	blood	88 901	81 935	756 652 ± 5 086	741 337 ± 57 954	65 811	56 040



**Fig. 3.** The rarefaction curves of TCRAD and TCRB diversities for each sample. The thymic samples are marked in red and peripheral samples in blue. The dots indicate observed diversity and counts, solid lines interpolated and dashed lines extrapolated values.



**Fig. 4.** Clustering of the samples on multi-dimensional scaling according to pairwise repertoire overlap comparison with Jaccard index.

“CalcDiversityStats” and “RarefactionPlot” commands with default settings to calculate and visualise diversity estimations, and finally “CalcPairwiseDistances” command to calculate the sequence overlap between two samples. For sequence overlap we selected the setting “strict”,

Table 4  
Overlap measures.

TCRAD				Counts of overlapping clonotypes in sample 1		Counts of overlapping clonotypes in sample 2		Pearson correlation	Relative overlap measure	Jaccard index	Morisita-Horn index	
Sample 1 id	Sample 2 id	Diversity sample 1	Diversity sample 2	Number of overlapping clonotypes	Counts sample 1	Counts sample 2	in sample 1	in sample 2				
thymus A	thymus B	6 763 870	7 419 245	866 679	11 838 086	12 849 473	3 401 700	3 572 924	0.881	1.73E-08	0.0051	0.904
thymus A	thymus C	6 763 870	5 259 057	630 350	11 838 086	8 359 283	2 823 352	2 076 828	0.844	1.77E-08	0.0553	0.876
thymus A	thymus D	6 763 870	6 610 182	773 916	11 838 086	11 063 464	3 177 831	2 897 003	0.853	1.73E-08	0.0614	0.882
thymus A	thymus 1	6 763 870	1 984 292	287 646	11 838 086	3 179 774	1 679 632	658 811	0.655	2.14E-08	0.0340	0.727
thymus A	thymus 2	6 763 870	1 198 677	177 662	11 838 086	1 747 487	1 213 379	332 560	0.600	2.19E-08	0.0228	0.673
thymus A	thymus 3	6 763 870	1 230 436	189 717	11 838 086	2 158 043	1 277 978	442 972	0.604	2.28E-08	0.0243	0.675
thymus A	thymus 4	6 763 870	1 345 927	203 596	11 838 086	1 848 851	1 353 803	361 818	0.633	2.24E-08	0.0258	0.702
thymus A	blood 1	6 763 870	130 307	29 044	11 838 086	154 682	316 005	37 176	0.251	3.30E-08	0.0042	0.218
thymus A	blood 2	6 763 870	103 142	20 600	11 838 086	123 523	223 783	26 054	0.148	2.95E-08	0.0030	0.151
thymus A	blood 3	6 763 870	170 333	34 574	11 838 086	245 126	347 204	57 652	0.014	3.00E-08	0.0050	0.004
thymus A	blood 4	6 763 870	157 728	28 303	11 838 086	199 326	300 479	38 946	0.042	2.65E-08	0.0041	0.048
thymus B	thymus C	7 419 245	5 259 057	657 775	12 849 473	8 359 283	3 031 445	2 129 578	0.837	1.69E-08	0.0547	0.870
thymus B	thymus D	7 419 245	6 610 182	808 433	12 849 473	11 063 464	3 416 024	2 969 838	0.851	1.65E-08	0.0611	0.882
thymus B	thymus 1	7 419 245	1 984 292	297 894	12 849 473	3 179 774	1 791 741	676 485	0.646	2.02E-08	0.0327	0.719
thymus B	thymus 2	7 419 245	1 198 677	184 222	12 849 473	1 747 487	1 292 592	342 539	0.586	2.07E-08	0.0218	0.663
thymus B	thymus 3	7 419 245	1 230 436	196 494	12 849 473	2 158 043	1 360 763	455 236	0.588	2.15E-08	0.0232	0.661
thymus B	thymus 4	7 419 245	1 345 927	210 731	12 849 473	1 848 851	1 438 108	371 861	0.619	2.11E-08	0.0246	0.693
thymus B	blood 1	7 419 245	130 307	29 717	12 849 473	154 682	330 377	37 934	0.246	3.07E-08	0.0040	0.206
thymus B	blood 2	7 419 245	103 142	21 239	12 849 473	123 523	236 641	26 746	0.151	2.78E-08	0.0028	0.145
thymus B	blood 3	7 419 245	170 333	35 705	12 849 473	245 126	366 684	57 206	0.022	2.83E-08	0.0047	0.008
thymus B	blood 4	7 419 245	157 728	29 322	12 849 473	199 326	316 077	40 215	0.041	2.51E-08	0.0039	0.046
thymus C	thymus D	5 259 057	6 610 182	596 367	8 359 283	11 063 464	2 003 982	2 481 328	0.827	1.72E-08	0.0529	0.863
thymus C	thymus 1	5 259 057	1 984 292	231 423	8 359 283	3 179 774	1 073 774	557 955	0.639	2.22E-08	0.0330	0.722
thymus C	thymus 2	5 259 057	1 198 677	147 145	8 359 283	1 747 487	794 176	283 569	0.598	2.33E-08	0.0233	0.686
thymus C	thymus 3	5 259 057	1 230 436	160 296	8 359 283	2 158 043	853 824	388 406	0.614	2.48E-08	0.0253	0.695
thymus C	thymus 4	5 259 057	1 345 927	169 687	8 359 283	1 848 851	897 840	311 017	0.640	2.40E-08	0.0264	0.719
thymus C	blood 1	5 259 057	130 307	24 337	8 359 283	154 682	266 500	31 520	0.238	3.55E-08	0.0045	0.229
thymus C	blood 2	5 259 057	103 142	17 448	8 359 283	123 523	150 315	22 968	0.137	3.22E-08	0.0038	0.154
thymus C	blood 3	5 259 057	170 333	29 699	8 359 283	245 126	233 789	46 909	0.072	3.32E-08	0.0055	0.070
thymus C	blood 4	5 259 057	157 728	24 276	8 359 283	199 326	204 214	33 909	0.047	2.93E-08	0.0045	0.049
thymus D	thymus 1	6 610 182	1 984 292	279 803	11 063 464	3 179 774	1 506 996	648 316	0.684	2.13E-08	0.0337	0.748
thymus D	thymus 2	6 610 182	1 198 677	174 157	11 063 464	1 747 487	1 089 914	327 307	0.621	2.20E-08	0.0228	0.689
thymus D	thymus 3	6 610 182	1 230 436	182 717	11 063 464	2 158 043	1 128 178	431 118	0.623	2.25E-08	0.0239	0.686
thymus D	thymus 4	6 610 182	1 345 927	196 751	11 063 464	1 848 851	1 200 784	351 610	0.655	2.21E-08	0.0254	0.719
thymus D	blood 1	6 610 182	130 307	28 731	11 063 464	154 682	287 455	36 894	0.268	3.34E-08	0.0043	0.217
thymus D	blood 2	6 610 182	103 142	20 581	11 063 464	123 523	211 785	26 154	0.169	3.02E-08	0.0031	0.156
thymus D	blood 3	6 610 182	170 333	33 661	11 063 464	245 126	313 960	54 297	0.023	2.99E-08	0.0050	0.007
thymus D	blood 4	6 610 182	157 728	27 545	11 063 464	199 326	269 325	38 363	0.067	2.64E-08	0.0041	0.052
thymus 1	thymus 2	1 984 292	1 198 677	84 060	3 179 774	1 747 487	252 989	176 256	0.545	3.53E-08	0.0271	0.749
thymus 1	thymus 3	1 984 292	1 230 436	86 181	3 179 774	2 158 043	256 879	230 215	0.652	3.53E-08	0.0275	0.752
thymus 1	thymus 4	1 984 292	1 345 927	91 687	3 179 774	1 848 851	270 795	184 905	0.657	3.43E-08	0.0283	0.758
thymus 1	blood 1	1 984 292	130 307	16 192	3 179 774	154 682	70 223	21 396	0.341	6.26E-08	0.0077	0.310
thymus 1	blood 2	1 984 292	103 142	11 184	3 179 774	123 523	50 623	14 471	0.245	5.46E-08	0.0054	0.248
thymus 1	blood 3	1 984 292	170 333	17 226	3 179 774	245 126	72 795	30 442	0.026	5.24E-08	0.0083	0.008
thymus 1	blood 4	1 984 292	157 728	14 345	3 179 774	199 326	60 045	19 682	0.137	4.58E-08	0.0067	0.215
thymus 2	thymus 3	1 198 677	1 230 436	57 752	1 747 487	2 158 043	128 317	166 052	0.625	3.92E-08	0.0244	0.722
thymus 2	thymus 4	1 198 677	1 345 927	62 995	1 747 487	1 848 851	138 699	196 036	0.641	3.90E-08	0.0254	0.728
thymus 2	blood 1	1 198 677	130 307	10 777	1 747 487	154 682	31 781	14 595	0.317	6.90E-08	0.0082	0.333
thymus 2	blood 2	1 198 677	103 142	8 069	1 747 487	123 523	23 923	10 565	0.229	6.53E-08	0.0062	0.274
thymus 2	blood 3	1 198 677	170 333	11 853	1 747 487	245 126	32 976	18 846	0.253	5.81E-08	0.0087	0.310
thymus 2	blood 4	1 198 677	157 728	10 266	1 747 487	199 326	29 087	14 063	0.260	5.43E-08	0.0076	0.374
thymus 3	thymus 4	1 230 436	1 345 927	66 420	2 158 043	1 848 851	186 971	141 677	0.630	4.01E-08	0.0265	0.761
thymus 3	blood 1	1 230 436	130 307	11 134	2 158 043	154 682	42 595	15 018	0.320	6.94E-08	0.0082	0.356
thymus 3	blood 2	1 230 436	103 142	7 922	2 158 043	123 523	31 210	10 341	0.217	6.24E-08	0.0060	0.289
thymus 3	blood 3	1 230 436	170 333	13 436	2 158 043	245 126	48 717	27 106	0.093	6.41E-08	0.0097	0.604
thymus 3	blood 4	1 230 436	157 728	10 754	2 158 043	199 326	39 869	14 765	0.168	5.54E-08	0.0078	0.309
thymus 4	thymus 1	1 345 927	130 307	11 729	1 848 851	154 682	32 338	15 699	0.296	6.69E-08	0.0080	0.308
thymus 4	thymus 2	1 345 927	103 142	8 364	1 848 851	123 523	23 731	10 893	0.182	6.02E-08	0.0058	0.233
thymus 4	thymus 3	1 345 927	170 333	13 389	1 848 851	245 126	36 254	21 997	0.075	5.84E-08	0.0089	0.064
thymus 4	blood 1	1 345 927	157 728	11 776	1 848 851	199 326	32 772	16 401	0.061	5.55E-08	0.0079	0.081
blood 1	blood 2	130 307	103 142	2 210	154 682	123 523	3 440	3 269	0.329	1.64E-07	0.0096	0.689
blood 1	blood 3	130 307	170 333	3 425	154 682	245 126	5 091	6 453	0.355	1.54E-07	0.0115	0.663
blood 1	blood 4	130 307	157 728	2 625	154 682	199 326	3 937	4 343	0.078	1.28E-07	0.0092	0.217
blood 2	blood 3	103 142	170 333	2 564	123 523	245 126	3 985	5 130	0.268	1.46E-07	0.0095	0.478
blood 2	blood 4	103 142	157 728	2 037	123 523	199 326	3 335	4 681	0.614	1.25E-07	0.0079	0.408
blood 3	blood 4	170 333	157 728	3 028	245 126	199 326	6 058	6 352	0.141	1.13E-07	0.0093	0.132

(continued on next page)

which requires matching CDR3 nucleotide regions as well as matching V genes and J genes. For visualisation of Jaccard index overlap values we used "ClusterSamples" tool that provides a multi-dimensional scaling plot created with isoMDS() function of MASS package for R.

Ethics Statement

The study was approved by the Pediatric Ethical Committee of the Helsinki University Hospital (HUS/747/2019) and a written informed consent was obtained from the parents.

Table 4 (continued)

TCRB																			
		Diversity		Number of		Counts		Counts of		Counts of		Pearson		Relative overlap		Morisita-Horn			
Sample 1 id	Sample 2 id	sample 1	sample 2	overlapping	clonotypes	sample 1	sample 2	overlapping	clonotypes	in sample 1	in sample 2	clonotypes	clonotypes	correlation	measure	Jaccard index	Index		
thymus A	thymus B	1 245 029	1 526 694	3 244	1 647 656	1 783 878	5 295	5 045	0.326	1.70E-09	0.00117	0.715							
thymus A	thymus C	1 245 029	1 551 603	2 545	1 647 656	1 850 299	4 108	3 863	0.266	1.30E-09	0.00091	0.737							
thymus A	thymus D	1 245 029	1 449 881	2 939	1 647 656	1 726 796	4 752	4 385	0.277	1.60E-09	0.00109	0.741							
thymus A	thymus 1	1 245 029	222 925	529	1 647 656	237 063	965	627	0.240	1.90E-09	0.00036	0.370							
thymus A	thymus 2	1 245 029	172 746	301	1 647 656	182 356	509	345	0.204	1.40E-09	0.00021	0.293							
thymus A	thymus 3	1 245 029	137 920	252	1 647 656	142 903	431	278	0.026	1.50E-09	0.00018	0.230							
thymus A	thymus 4	1 245 029	121 483	221	1 647 656	128 228	373	249	0.063	1.50E-09	0.00016	0.208							
thymus A	blood 1	1 245 029	77 281	555	1 647 656	82 418	929	694	0.246	5.80E-09	0.00042	0.110							
thymus A	blood 2	1 245 029	69 404	361	1 647 656	73 945	632	433	-0.018	4.20E-09	0.00027	0.096							
thymus A	blood 3	1 245 029	103 551	550	1 647 656	134 110	1 023	651	0.109	4.30E-09	0.00041	0.216							
thymus A	blood 4	1 245 029	81 935	299	1 647 656	88 901	545	346	0.015	2.90E-09	0.00023	0.138							
thymus B	thymus C	1 526 694	1 551 603	2 919	1 783 878	1 850 299	4 371	4 373	0.280	1.20E-09	0.00095	0.740							
thymus B	thymus D	1 526 694	1 449 881	3 357	1 783 878	1 726 796	5 097	4 974	0.345	1.50E-09	0.00113	0.774							
thymus B	thymus 1	1 526 694	222 925	597	1 783 878	237 063	997	700	0.339	1.80E-09	0.00034	0.335							
thymus B	thymus 2	1 526 694	172 746	340	1 783 878	182 356	562	375	0.134	1.30E-09	0.00020	0.282							
thymus B	thymus 3	1 526 694	137 920	319	1 783 878	142 903	541	354	0.042	1.50E-09	0.00019	0.213							
thymus B	thymus 4	1 526 694	121 483	290	1 783 878	128 228	497	325	0.085	1.60E-09	0.00018	0.198							
thymus B	blood 1	1 526 694	77 281	615	1 783 878	82 418	1 035	762	0.181	5.20E-09	0.00038	0.100							
thymus B	blood 2	1 526 694	69 404	401	1 783 878	73 945	679	458	0.119	3.80E-09	0.00025	0.112							
thymus B	blood 3	1 526 694	103 551	623	1 783 878	134 110	1 056	769	0.118	3.90E-09	0.00038	0.163							
thymus B	blood 4	1 526 694	81 935	349	1 783 878	88 901	578	401	0.052	2.80E-09	0.00022	0.124							
thymus C	thymus D	1 551 603	1 449 881	3 300	1 850 299	1 726 796	5 015	4 900	0.244	1.50E-09	0.00110	0.750							
thymus C	thymus 1	1 551 603	222 925	671	1 850 299	237 063	1 110	803	0.251	1.90E-09	0.00038	0.307							
thymus C	thymus 2	1 551 603	172 746	387	1 850 299	182 356	674	452	0.204	1.40E-09	0.00022	0.266							
thymus C	thymus 3	1 551 603	137 920	280	1 850 299	142 903	471	314	0.061	1.30E-09	0.00017	0.199							
thymus C	thymus 4	1 551 603	121 483	269	1 850 299	128 228	439	307	0.060	1.40E-09	0.00016	0.174							
thymus C	blood 1	1 551 603	77 281	752	1 850 299	82 418	1 283	983	0.253	6.30E-09	0.00046	0.094							
thymus C	blood 2	1 551 603	69 404	468	1 850 299	73 945	779	559	0.057	4.30E-09	0.00029	0.096							
thymus C	blood 3	1 551 603	103 551	702	1 850 299	134 110	1 252	887	0.192	4.40E-09	0.00042	0.160							
thymus C	blood 4	1 551 603	81 935	330	1 850 299	88 901	585	387	0.090	2.60E-09	0.00020	0.130							
thymus D	thymus 1	1 449 881	222 925	656	1 726 796	237 063	1 048	786	0.279	2.00E-09	0.00039	0.315							
thymus D	thymus 2	1 449 881	172 746	386	1 726 796	182 356	632	445	0.120	1.50E-09	0.00024	0.269							
thymus D	thymus 3	1 449 881	137 920	316	1 726 796	142 903	524	360	0.015	1.60E-09	0.00020	0.206							
thymus D	thymus 4	1 449 881	121 483	276	1 726 796	128 228	412	311	0.036	1.60E-09	0.00018	0.176							
thymus D	blood 1	1 449 881	77 281	711	1 726 796	82 418	1 173	935	0.072	6.30E-09	0.00047	0.088							
thymus D	blood 2	1 449 881	69 404	412	1 726 796	73 945	692	466	0.126	4.10E-09	0.00027	0.117							
thymus D	blood 3	1 449 881	103 551	718	1 726 796	134 110	1 199	897	0.120	4.80E-09	0.00046	0.152							
thymus D	blood 4	1 449 881	81 935	386	1 726 796	88 901	600	472	0.055	3.20E-09	0.00025	0.094							
thymus 1	thymus 2	222 925	172 746	68	237 063	182 356	92	82	0.165	1.80E-09	0.00017	0.748							
thymus 1	thymus 3	222 925	137 920	75	237 063	142 903	98	86	0.059	2.40E-09	0.00021	0.749							
thymus 1	thymus 4	222 925	121 483	47	237 063	128 228	55	55	-0.048	1.70E-09	0.00014	0.750							
thymus 1	blood 1	222 925	77 281	301	237 063	82 418	371	464	0.097	1.75E-08	0.00100	0.292							
thymus 1	blood 2	222 925	69 404	99	237 063	73 945	118	122	0.153	6.40E-09	0.00034	0.463							
thymus 1	blood 3	222 925	103 551	192	237 063	134 110	243	236	0.284	8.30E-09	0.00059	0.741							
thymus 1	blood 4	222 925	81 935	63	237 063	88 901	85	78	0.032	3.40E-09	0.00021	0.488							
thymus 2	thymus 3	172 746	137 920	32	182 356	142 903	40	40	0.000	1.30E-09	0.00010	0.848							
thymus 2	thymus 4	172 746	121 483	35	182 356	128 228	40	42	0.000	1.70E-09	0.00012	0.836							
thymus 2	blood 1	172 746	77 281	92	182 356	82 418	104	112	0.074	6.90E-09	0.00037	0.590							
thymus 2	blood 2	172 746	69 404	77	182 356	73 945	88	102	0.051	6.40E-09	0.00032	0.405							
thymus 2	blood 3	172 746	103 551	99	182 356	134 110	114	3 222	-0.049	5.50E-09	0.00036	0.001							
thymus 2	blood 4	172 746	81 935	42	182 356	88 901	49	48	0.183	3.00E-09	0.00016	0.741							
thymus 3	thymus 4	137 920	121 483	29	142 903	128 228	33	30	-0.060	1.70E-09	0.00011	0.916							
thymus 3	blood 1	137 920	77 281	76	142 903	82 418	89	104	0.214	7.10E-09	0.00035	0.591							
thymus 3	blood 2	137 920	69 404	46	142 903	73 945	49	49	-0.041	4.80E-09	0.00022	0.745							
thymus 3	blood 3	137 920	103 551	126	142 903	134 110	153	5 923	0.042	8.80E-09	0.00052	0.001							
thymus 3	blood 4	137 920	81 935	40	142 903	88 901	47	43	-0.062	3.50E-09	0.00018	0.774							
thymus 4	blood 1	121 483	77 281	44	128 228	82 418	50	53	0.065	4.70E-09	0.00022	0.672							
thymus 4	blood 2	121 483	69 404	46	128 228	73 945	49	64	-0.009	5.50E-09	0.00024	0.341							
thymus 4	blood 3	121 483	103 551	53	128 228	134 110	66	71	0.181	4.20E-09	0.00024	0.772							
thymus 4	blood 4	121 483	81 935	81	128 228	88 901	92	137	0.112	8.10E-09	0.00040	0.369							
blood 1	blood 2	77 281	69 404	195	82 418	73 945	272	240	0.045	3.64E-08	0.00133	0.736							
blood 1	blood 3	77 281	103 551	328	82 418	134 110	496	448	0.303	4.10E-08	0.00182	0.656							
blood 1	blood 4	77 281	81 935	126	82 418	88 901	159	156	0.219	1.99E-08	0.00079	0.825							
blood 2	blood 3	69 404	103 551	173	73 945	134 110	219	218	0.108	2.41E-08	0.00100	0.665							
blood 2	blood 4	69 404	81 935	87	73 945	88 901	114	111	0.032	1.53E-08	0.00058	0.737							
blood 3	blood 4	103 551	81 935	130	134 110	88 901	175	158	0.102	1.53E-08	0.00070	0.704							

CRedit Author Statement

NH and TPA conceptualised the study and wrote the original manuscript. NH and RV collected and prepared the samples. IK, DAY and JS implemented the software usage. IPM provided the study material. All authors reviewed and accepted the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships which have, or could be perceived to have, influenced the work reported in this article.

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